GEORGIA INSTITUTE OF TECHNOLOGY — OFFICE OF CONTRACT ADMINISTRATION

PROJECT ADMINISTRATION DATA SHEET

[X] ORIGINAL [ ] REVISION NO. ____

Project No. G33-401 NIH — DATE 9-21-82

Project Director: Dr James C. Powers — School/~~Lab~~ Chemistry

Sponsor: DHHS/PHS/NIH — National Heart, Lung and Blood Institute

Type Agreement: Grant No. 1-R01-HL29307-01

Award Period: From 8-1-82 To 7-31-83 (Performance) 10-31-83 (Reports)

Sponsor Amount: $ 85,017 — Contracted through: ~~GTRC~~ GIT

Cost Sharing: $ 4,474 (G33-341)

Title: Synthetic Elastase Inhibitors

ADMINISTRATIVE DATA — OCA Contact Don Hasty

1) Sponsor Technical Contact:

Dr. Z. Bengali
Division of Lung Diseases
National Heart, Lung + Blood Institute
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Defense Priority Rating: N/A

2) Sponsor Admin/Contractual Matters:

Ms Robin Bissell
Grants Operations Branch
Division of Extramural Affairs
National Heart, Lung + Blood Institute
Bethesda, Md 20205
Phone (301) 496-7255

Security Classification: N/A

RESTRICTIONS

See Attached NIH Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval – Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

Equipment: Title vests with GIT. We are accountable for all equipment purchased.

COMMENTS: First year of this grant.

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FORM OCA 4:781

N-7-8 2-SR 306

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date 10/12/83

Project No. G-33-U01 School/~~xxx~~ Chemistry

Includes Subproject No.(s) NONE

Project Director(s) Dr. James C. Powers. ~~GTRI~~ GIT

Sponsor HHS/PHS/NIH - National Heart, Lung, and Blood Institute.

Title: Synthetic Elastase Inhibitors

Effective Completion Date: 7/31/83 (Performance) _____ (Reports)

Grant/Contract Closeout Actions Remaining:

- [X] None
- [ ] Final Invoice or Final Fiscal Report
- [ ] Closing Documents
- [ ] Final Report of Inventions
- [ ] Govt. Property Inventory & Related Certificate
- [ ] Classified Material Certificate
- [ ] Other _____

Continues Project No. _____

Continued by Project No. G-33-U02

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Form OCA 58.1004

SECTION IV PROGRESS REPORT SUMMARY	GRANT NUMBER HL 29307-02	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR POWERS, James C.	PERIOD COVERED BY THIS REPORT FROM	THROUGH
NAME OF ORGANIZATION Georgia Institute of Technology	8/1/82	5/17/83

TITLE *(Repeat title shown in item 1 on first page)*
Synthetic Elastase Inhibitors

(SEE INSTRUCTIONS)

Publications

The following publications were supported by HL 18679, which is the precursor to this grant.

"Proteolytic Enzymes and Their Active-Site Specific Inhibitors: Role in the Treatment of Disease," Powers, J.C.(1982) Advances in Chemistry 198 , 347-367.

"A New Class of Heterocyclic Serine Protease Inhibitors. Inhibition of Human Leukocyte Elastase, Porcine Pancreatic Elastase, Cathepsin G, and Bovine Chymotrypsin A with Substituted Benzoxazinones, Quinazolines, and Anthranilates," Teshima, T., Griffin, J.C., and Powers, J.C.(1982) J Biol. Chem. 257 , 5085-5091.

"Synthetic Elastase Inhibitors: Prospects for Use in the Treatment of Emphysema", Powers, J.C.(1983) Am. Rev. Respir. Dis. 127 , S54-S58.

Publication Submitted

"New Mechanism Based Serine Protease Inhibitors: Inhibition of Human Leukocyte Elastase, Porcine Pancreatic Elastase, Human Leukocyte Cathepsin G, and Chymotrypsin by 3-Chloroisocoumarin and 3,3-Dichlorophthalide," Harper, J.W., Hemmi, K., and Powers, J.C.(1983) J. Am. Chem. Soc. , submitted.

Progress Report

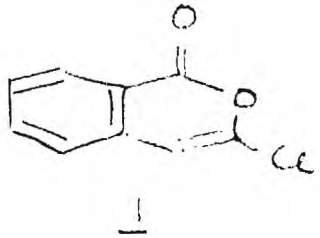
Scientific Goals. Proteolysis of lung elastin and other connective tissue proteins released from leukocytes is generally thought to cause the destruction of the lung which is observed in pulmonary emphysema. The two major serine proteases of leukocytes, human leukocyte elastase and cathepsin G, will hydrolyze lung elastin. In addition to emphysema, elastase is involved in amyloidosis and chronic inflammation, while pancreatic elastase is involved in pancreatitis. Cathepsin G and other chymotrypsin-like enzymes from mast cells are speculated to play a role in inflammation and arthritis.

The goal of this research is to develop an elastase inhibitor which would be useful for the treatment of human emphysema. A variety of structures will be investigated including heterocyclic structures which may be transition state analogs, heterocyclic mechanism based inhibitors, peptide analogs, and selective acylating agents. All the inhibitors will be tested with cathepsin G and other chymotrypsin-like enzymes. Any promising inhibitors will be provided to other investigators for studies in animal models of emphysema. This research should lead to a better understanding of active site structures of the enzymes involved in connective tissue turnover and should lead to new theapeutic methods for the treatment of pulmonary emphysema.

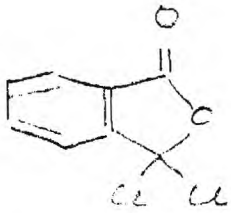
Progress. During the first year of this grant, we have discovered a new mechanism based elastase inhibitor which I believe has considerable potential for use in treatment of human emphysema. At present I have discerned no negative features to this inhibitor and one of our next steps will be to encourage other investigators to test this inhibitor in animals. The inhibitor is 3-chloroisocoumarin.

Mechanism based irreversible inhibitors, which have been reported for porcine

pancreatic (PP) elastase and bovine pancreatic chymotrypsin A , include 6-halomethylcoumarins, nitroso lactams, haloenol lactones, isatoic anhydride and 3H-1,3-oxazine-2,6-diones. Human leukocyte (HL) elastase and cathepsin G are inhibited reversibly by heterocyclic structures such as 2-substituted-4H-3,1-benzoxazin-4-ones and N-arylbenzisothiazolinone 1,1-dioxides. This suggested that heterocyclics containing masked reactive functionalities might act as mechanism based irreversible inhibitors for HL elastase and cathepsin G, enzymes for which no such inhibitors has been demonstrated. Therefore we prepared 3-chloroisocoumarin (1) and 3,3-dichlorophthalide (2) and have found them to be potent irreversible inhibitors of several serine proteases.



1



2

Incubation of 1 and 2 with HL elastase, PP elastase, and chymotrypsin A resulted in a rapid time dependent inhibition of enzyme activity. Cathepsin G was only inhibited by 2 , and 1 did not inhibit trypsin or the cysteine protease papain. In all cases the inhibition rate was dependent upon inhibitor concentration. Rates of inhibition of HL and PP elastase were decreased dramatically when the reversible inhibitors 2-pentafluoropropyl-4H-3,1-benzoxazin-4-one and CF_3CO-Lys-Ala-$NHC_6H_4CH_3$ were added respectively to the incubation solutions, indicating that the inhibitors are active site directed.

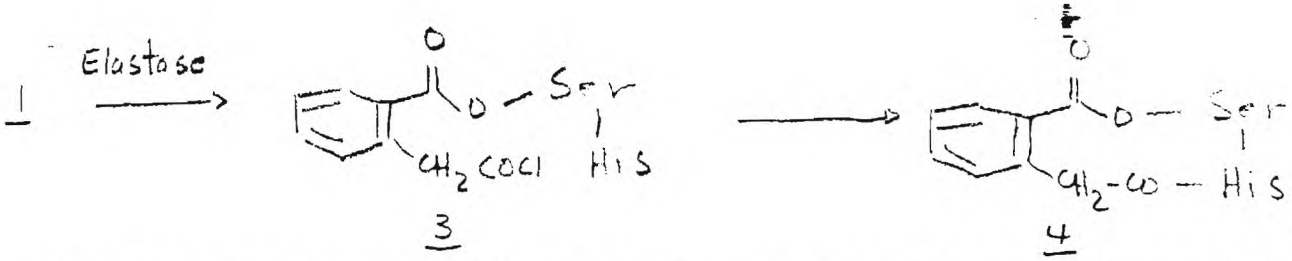
HL and PP elastase inhibited by 1 were quite stable to reactivation upon standing, while cathepsin G, chymotrypsin and all the enzymes inhibited with 2 regained activity upon standing. The presence of labile acyl moieties in the inhibited protease was also indicated by the rapid reactivation (< 4 min) of HL and PP elastase inactivated by 1 when buffered hydrazine (1-3 mM) was added.

Hydrolysis of 1 to 2-carboxyphenylacetic acid (homophthalic acid) in the presence and absence of enzyme could be monitored by an absorbance decrease at 325 nm. The rate increased when enzyme was added indicating that the enzyme was turning over the inhibitor. This is a characteristic of mechanism based inhibitors. Other studies suggested >15 turnovers/inactivation for HL elastase and >4 for PP elastase. The reaction of 1 with chymotrypsin A is almost stoichiometric since 1.0 equivalent of 1 resulted in 90 % inhibition.

Reaction of 1 with chymotrypsin A in aqueous solution utilizing a pH stat resulted in the rapid release of 0.92 equivalents of protons after 6 min at which time the residual enzymatic activity was 9.8 %. Similar experiment with 2 and chymotrypsin A resulted in the release of 2.7 equivalents of protons after 15 min at which time the enzymatic activity was 8 %. Further incubation resulted in the release of 3.1 total protons with 1 (theoretical 3) after 115 min. At pH 8.5 under identical conditions, 1 reacted with chymotrypsin A to release 0.95 protons within 3 min and slowly released the additional protons over the course of 1 hr.

The above results are consistent with the following scheme where serine proteases react with 3-chloroisocoumarin 1 with the formation of the acyl enzyme 3 by reaction at the active site serine residue. Formation of the diacylated product 4 then occurs by reaction at histidine-57, the only likely nucleophile in the active site of most serine proteases. The alternate structure in which a protonated histidine interacts with the inhibitor to form a stabilized acyl enzyme can be ruled out by the pH stat results. Only one proton would be released at pH 7.5 if the pK of the histidine was increased due

to a favorable electrostatic interaction with the inhibitor carboxyl group. However at higher pHs, one would expect 2 protons to be released and this was not observed.



The evidence indicates that 1 and 2 are mechanism based irreversible inhibitors of serine proteases. These are the first demonstrated examples of enzyme activated inhibitors of HL elastase and cathepsin G. These enzymes have been noted to be major contributors to elastin destruction observed in emphysema. These inhibitors and similar structures may have considerable pharmacologic potential as inhibitors *in vivo* .

Specific Objectives for Next Year. Studies leading to a clearer understanding of the inhibition processes involved in the reaction of HL elastase with 3-chlorocoumarin are now in progress. We are testing the solublity and stablity of 3-chlorocoumarin in preparation for getting it tested in animals. We will also test the reactivity of 3-chloroisocoumarin toward nucleophiles such as glutathione and its life-time in plasma. At the same time, we are trying to extend this lead by synthesizing other structures which are capable of reacting with HL elastase releasing reactive functional groups. In particular, we are examining structures which contain masked chloromethyl ketone functional groups. When this group is unmasked, it could potentially alkylate the histidine of elastase.

We are also cooperating with Dr. Edgar Meyer at Texas A & M on an x-ray study of structure of PP elastase inhibited by 3-chloroisocoumarin. He plans to collect a data set on the enzyme inhibitor complex during the summer while he is in Huber's lab in Germany. This fall, I plan to make a trip to Texas to do some modeling in Dr. Meyer's lab. No x-ray structure on HL elastase is yet available. However, modeling with the structure of the closely related enzyme PP elastase often yields valuable insights for the design of HL elastase inhibitors.

Since we have spent all of our recent effort on the mechanism based inhibitors, we have not yet started some of the other problems which we orginally proposed. However, I have a new postdoc from Japan (Dr. Hori) starting in August. He will probably work either on the acylating agents for elastase or the peptide transition state inhibitors which we described in the orginal proposal.